• 計畫中文名稱	Notch1 受體訊號傳遞路徑在癌細胞所扮演角色之探討		
• 計畫英文名稱	Study the Roles of the Notch1 Signaling in Cancer Cells		
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• 英文關鍵字	Notch receptor		
• 中文摘要	Notch 蛋白爲細胞表面的受體，在結構上具有高度保留性，對於細胞命運的決定，以及前驅細胞數量、特性的保持，都扮演著相當重要的角色。隨著細胞種類的不同， Notch 受體的活化作用參與有關細胞分化、增生與凋亡的不同調控角色。許多參與 Notch 訊號傳遞路徑的基因之異常現象，在人類的 leukemias、Alagille 及 CADASIL syndromes 都被發現，因此 Notch 受體訊號傳遞路徑的異常與人類疾病的造成有關。 目前已知的 Notch 受體訊號傳遞路徑爲：藉由與相鄰細胞的 ligand 結合，Notch 受體被活化而導致此蛋白被分解，此時 Notch 受體細胞內區域被釋出，並且移動到細胞核中，與核內細胞因子作用，促使訊號往下傳遞。藉由或不藉由轉錄因子 CBF1 的兩種方式，Notch 受體細胞內區域均可活化下游基因的表現。 Notch 訊號傳遞路徑的調控相當複雜，截至目前仍混沌不清。雖然已發現一些 Notch1 受體細胞內區域 (N1IC) 的結合蛋白，位於細胞中不同的位址，活化或抑制 Notch 訊號傳遞路徑，但仍無法得知詳細的分子調控機制。本實驗室於先前已進行 N1IC 的結合蛋白之篩選及鑑定工作，研究發現：在人類 Jurkat 以及 T-cell acute lymphoblastic leukemias (T-ALL) 兩種細胞內，內生性的 Notch1 受體細胞內區域都會與細胞內轉錄因子 YY1 結合，而且在細胞核中形成很大的 complex。更進一步研究顯示：N1IC 可活化具有 CBF1-response elements 的 promoter activity，經由此結合作用，這個活化現象會被轉錄因子 YY1 所抑制。N1IC 藉由與 CBF1 蛋白結合，而可以結合在 CBF1-response elements 的 DNA 上；轉錄因子 YY1 也藉由與 N1IC 結合，而間接結合在此 DNA 上。 延續這些研究結果，本計畫擬更進一步探討 Notch 訊號傳遞路徑。首先擬分析細胞核中，包含轉錄因子 YY1 的這個大的 Notch complex 之組成，藉由了解結合在此大的 Notch1 complex 的細胞因子，探討這些細胞因子所扮演的角色及其生物功能。爲了探討這個大的 Notch complex 在細胞核中所扮演的角色，同時也將藉由 chromatin immunoprecipitation (ChIP) 的分析方法，篩選細胞內經由此大的 Notch complex 結合所調控的基因。同時也將檢視此大的 Notch complex 對這些所篩選到的基因之基因表現、生物功能的影響。 除了轉錄因子 YY1 之外，本實驗		

	室先前也篩選到數個其他的 N1IC 結合蛋白，例如：βII-tubulin。除了擬分析這些結合現象之外，在本研究計畫中也將進一步探討這些結合作用所扮演的功能及角色。此外，在前面的研究中，本實驗室也發現造血幹原/前趨細胞往紅血球系分化時，細胞內 b2-microglobulin 表現量會產生改變。在本研究計畫中也將評估於造血過程中，b2-microglobulin 對於 Notch 訊號傳遞路徑的影響。
• 英文摘要	Notch genes encode evolutionally conserved receptors that have been utilized to control cell fate decisions during development. Notch signaling participates in several cellular functions such as proliferation, apoptosis, and differentiation, depending upon the cellular context of Notch activation. Acquired or inherited abnormalities in genes involving in Notch signaling have been found in human leukemias and Alagille and CADASIL syndromes. Therefore, perturbations of Notch signal pathway underlie several forms of human disease. In the prevailing model for Notch signaling, Notch receptors are activated through binding with ligands on neighboring cells. Notch intracellular domains are released and translocated into nucleus after proteolytic cleavages triggered by ligand binding. Then Notch intracellular domains activate the expression of their target genes via both CBF1-dependent and ？HVindependent pathway. The control of Notch signaling is very complicated and not fully understood yet. So far, there are several Notch1 receptor intracellular domain (N1IC)-associating cellular factors that were identified to modulate the Notch signaling both positively and negatively. These data indicate that the activity of Notch signaling is modulated by different cellular factors in different subcellular compartments. Though the members of Notch-associated factors and the downstream target genes are expanding, the molecular mechanisms of Notch signaling in diverse developmental systems remain unsolved. In the previous study, we had screened and characterized the N1IC-associating proteins. Transcription factor Ying Yang 1 (YY1) was identified to associate with N1IC in the high-molecular-weight complexes in nuclei and this association modulated the CBF1-dependent gene expression. Furthermore, YY1 indirectly regulates the transcriptional activity of the wild-type CBF1 response elements via the direct interaction of N1IC and CBF1. We also demonstrated the association between endogenous N1IC and intrinsic YY1 in both human Jurkat cells and acute T cell lymphoblastic leukemia (T-ALL) cells. Taken together, these results indicated transcription factor YY1 may modulate Notch signaling via association with the large Notch complexes. To further study Notch signaling, we would like to dissect the components of the large Notch complex containing YY1 in nuclei. The biological functions of these components in this large complex will be clarified. To assess the roles of this large complex in nuclei, we will identify the cellular targeted genes of this large complex by chromatin immunoprecipitation assay (ChIP). Then we will further examine whether this large Notch complex regulates the expression and biological functions of these identified candidates. We had identified several N1IC-associating factors such as ? H]II-tubulin in the previous study. In this proposal, we will also examine the roles of the association between N1IC and these identified N1IC-associating factors. In addition, we found b2-microglobulin is expressed differentially during erythropoiesis of hematopoietic stem/progenitor cells. The biological functions of b2-microglobulin in Notch signaling will be investigated in this proposal.